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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: THE USE OF DIESTERS OF ASTAXANTHIN FOR ENHANCING THE GROWTH OF FARMED FISH

(57) Abstract: This invention relates to the use of diesters of astaxanthin prepared with omega-3 fatty acids and/or short chain carboxylic acids for enhancing the growth of farmed fish, as a growth-enhancing agent in feed for farmed fish, as an appetizer in feed for fish as well as for increasing the utilization of the feed for farmed fish, and for optimising health and well-being of farmed fish.



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## The use of diesters of astaxanthin for enhancing the growth of farmed fish

This invention relates to the use of diesters of astaxanthin prepared with omega-3 fatty acids and/or short chain carboxylic acids for enhancing the growth of farmed fish.

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In the aquaculture industry it is a continual focus on the growth of the fish. It is a goal to produce healthy fish that rapidly gain weight and thereby obtain a satisfying slaughtering weight in a minimum of time.

10 To improve on this goal a lot of effort is put in development of feed for farmed fish. However, there are still a need for more an optimised feed.

In Norwegian patent application No. 1999 1857 it is disclosed that diesters of astaxanthin prepared with omega-3 fatty acids and/or short chain carboxylic acids have  
15 excellent properties as a pigment in feed for salmonides.

Now the inventors of the present invention surprisingly have found that a feed comprising diesters of astaxanthin prepared with omega-3 fatty acids and/or short chain carboxylic acids exhibit unexpected results concerning the growth of salmonides. The  
20 person skilled in the art will realise that the growth-enhancing effects will be of great value also for fish species other than those where astaxanthin is given for pigmentation of the flesh. The present invention is intended for all fish species that are reared in aquaculture.

25 Thus, it is a main object of the present invention to provide a growth-enhancing agent for feed to farmed fish.

Another object of this invention is to provide a feed that improves the uptake of the feed in the fish.

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Still another object of this invention is to provide a feed that is more appetizing for the fish.

These and other objects are achieved by the attached claims.

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The invention is further explained by examples.

The person skilled in the art will know that improved growth is linked to improved health and well-being of the fish. Thus the present invention also provides a feed that gives an optimised health and well-being of farmed fish.

5

### Example 1

Diester of astaxanthin was prepared with a concentrate of omega-3 fatty acids comprising approximately 50% eicosapentaenoic acid (EPA) (all cis C20:5 n3) and  
10 approximately 35% docosahexaenoic acid (DHA) (all cis C22:6 n3).

The EPA/DHA astaxanthin diester (ACD) above (30 mg/kg calculated as free (i.e. unesterified) astaxanthin) was added during pilot plant production of fish fodder according to a standard recipe. The fat content of the fodder was approx. 35%. Urea  
15 (0.7% by weight) was added with water during extrudation. ACD was added together with fish oil by vacuum-coating of the extruded pellets. Several batches of this fodder composition were produced.

Commercial astaxanthin (Carophyll Pink, Roche, 30 mg/kg calculated as free  
20 astaxanthin) was added to fish fodder in the same manner as above. In this commercial product unesterified astaxanthin is finely dispersed in a starch-covered matrix of gelatine and carbohydrates. Ethoxyquin and ascorbyl palmitate are added as antioxidants. The process and raw materials, including urea addition, were identical with what is given above. Several batches of these fodder compositions were produced  
25 (one with and one without added urea).

Urea was added in order to study whether this had an effect on fodder quality. The calculations related to utilisation of feed (table 3) are not corrected for the small weight contribution of urea (0.7%).

30

The three fodder compositions were given to Atlantic salmon. Initially, the average weight of the fish was 68 grams for all groups. Initially, there was 800 fish in each group. After 11.5 months the number was reduced to approx. 300 fish in each group. The fish were fed manually *ad libido*. Table 1 and 2 below show the weight  
35 development in the different groups.

Table 1

Average weight (g):

<b>Time</b>	<b>Control</b>	<b>Control with urea</b>	<b>ACD</b>
8 months	1132	1153	1180
11.5 months	1345	1402	1437
15.5 months	3967	4078	4320

5 Table 2

Average weight relative to control:

<b>Time</b>	<b>Control</b>	<b>Control with urea</b>	<b>ACD</b>
8 months	1.00	1.02	1.04
11.5 months	1.00	1.04	1.07
15.5 months	1.00	1.03	1.09

10 Utilisation of feed

After 11.5 months the number of fish in each group was reduced to approx. 300. Table 3 below shows the weight increase related to fodder consumption:

Table 3

	<b>Control</b>	<b>Control with urea</b>	<b>ACD</b>
Average weight (kg)	3.967	4.078	4.320
Number of fish:	296	293	294
Increase of biomass (kg) from 11.5 months	776.1	784.1	847.6
Fodder consumption (kg) from 11.5 months	875.5	888.8	906.9
Increase of biomass/fodder (relative to control)	0.8865 (1.00)	0.8822 (1.00)	0.9347 (1.05)

The results show that ACD gives an increase of weight compared to that of the control. The increase is also higher than the control plus urea (Table 1 and 2). The increase of weight seems partly to be due to a better utilisation of the fodder. Addition of urea alone  
5 does not seem to have any effect in this respect (Table 3).

P a t e n t       c l a i m s

1.

Use of a diester of astaxanthin prepared with an omega-3 fatty acid and/or a short chain  
5 carboxylic acid for enhancing the growth of farmed fish.

2.

Use of a diester of astaxanthin prepared with an omega-3 fatty acid and/or a short chain  
carboxylic acid as a growth-enhancing agent in feed for farmed fish.

10

3.

Use of a diester of astaxanthin prepared with an omega-3 fatty acid and/or a short chain  
carboxylic acid as an appetizer in feed for farmed fish.

15

4.

Use of a diester of astaxanthin prepared with an omega-3 fatty acid and/or a short chain  
carboxylic acid for increasing the utilization of the feed for farmed fish.

5.

20 Use of a diester of astaxanthin prepared with an omega-3 fatty acid and/or a short chain  
carboxylic acid for optimising health and well-being of farmed fish.

6.

25 Use according to any of the claims 1-5, wherein the omega-3 fatty acid comprises a total  
amount of eicosapentaenoic acid (EPA) (all cis C20:5 n3) and/or docosahexaenoic acid  
(DHA) (all cis C22:6 n3) from 18 to 100 %.

7.

30 Use according to any of the claims 1-5, wherein the omega-3 fatty acid comprises a  
total amount of eicosapentaenoic acid (EPA) (all cis C20:5 n3) and/or docosahexaenoic  
acid (DHA) (all cis C22:6 n3) from 40 to 100 %.

8.

35 Use according to any of the claims 1-5, wherein the omega-3 fatty acid comprises an  
amount of eicosapentaenoic acid (EPA) (all cis C20:5 n3) from 8 to 98 % and/or an  
amount of docosahexaenoic acid (DHA) (all cis C22:6 n3) from 8 to 98 %.

9.

Use according to any of the claims 1-5, wherein the omega-3 fatty acid comprises an amount of eicosapentaenoic acid (EPA) (all cis C20:5 n3) from 25 to 98 % and/or an amount of docosahexaenoic acid (DHA) (all cis C22:6 n3) from 15 to 98 %.

5

10.

Use according to any of the claims 1-5, wherein the omega-3 fatty acid comprises approximately 50 % eicosapentaenoic acid (EPA) (all cis C20:5 n3) and approximately 35 % docosahexaenoic acid (DHA) (all cis C22:6 n3).

10

11.

Use according to any of the claims 1-5, wherein the short chain carboxylic acid contains from 1-12 carbon atoms.

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12.

Use according to claim 11, wherein the short chain carboxylic acid is formic acid.

13.

Use according to any of the preceding claims, wherein the farmed fish are salmonides.

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/NO 02/00246

## A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A23K 1/18, A23K 1/16

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI DATA, AQUASCI

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 0062625 A1 (NORSK HYDRO ASA), 26 October 2000 (26.10.00) --	1-13
X	Pure & Appl. Chem., Vol. 66, No 5, 1994, Samuel P. Meyers: "Developments in world aquaculture, feed formulations, and role of carotenoids", pages 1069-1076 --	1-13
X	Aquaculture, vol. 146, 1996, Trond Storebakken et al: "Plasma carotenoid concentration indicates the availability of dietary astaxanthin for Atlantic salmon, Salmo salar, pages 147-153 --	1-13

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

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## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Journal of Fish Diseases, vol. 18, 1995, R. Christiansen et al: "Antioxidant status and immunity in Atlantic salmon, Salmo salar L., fed semi-purified diets with and without astaxanthin supplementation", pages 317-328  --	1-13
A	Aquaculture and Fisheries Management, vol. 25, 1994, R. Christiansen et al: "Effect of astaxanthin and vitamin A on growth and survival during first feeding of Atlantic salmon, Salmo salar L." pages 903-914  --	1-13
A	Proc. Third Int. Symp. on Feeding and Nutr. in Fish Toba, Aug 28 - Sept 1, 1989, Ole J. Torrissen: "Biological Activities of Carotenoids in Fishes", pages 387-399  --	1-13
A	DATABASE WPI Derwent Publications Ltd., London, GB; AN 1991-145985 & JP 30 83577 A (DAINIPPON INK & CHEM KK), 9 April 1991 (1991-04-09) abstract  -- -----	1-13

### Information on patent family members

International application No.

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Form PCT/ISA/210 (patent family annex) (July 1998)